*The demand must be filed directly with the competent International Preliminary Examining Authority or, if two or more Authorities are competent, with the one chosen by the applicant. The full name or two-letter code of that Authority may be indicated by the applicant on the line below:*

**IPEA/** EP

CONFIRMATION COPY OF THE FAX OF
01 JUN 2005

# PCT

**CHAPTER II**

## DEMAND

under Article 31 of the Patent Cooperation Treaty:

The undersigned requests that the international application specified below be the subject of international preliminary examination according to the Patent Cooperation Treaty.

For International Preliminary Examining Authority use only

| Identification of IPEA | Date of receipt of DEMAND |
|---|---|
| | |

**Box No. I IDENTIFICATION OF THE INTERNATIONAL APPLICATION**

Applicant's or agent's file reference: T2952-PCT

| International application No. | International filing date *(day/month/year)* | (Earliest) Priority date *(day/month/year)* |
|---|---|---|
| PCT/BE2004/000117 | 12 August 2004 (12.08.2004) | 12 August 2003 (12.08.2003) |

Title of invention: Use of CXCL6 chemokine in the prevention or repair of cartilage defects

**Box No. II APPLICANT(S)**

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country.)*

TIGENIX N.V.
Technologielaan 3
B-3001 Leuven
Belgium

Telephone No.: +32 16 39 60 60
Facsimile No.: +32 16 39 60 70
Teleprinter No.:
Applicant's registration No. with the Office:

| State *(that is, country)* of nationality: | State *(that is, country)* of residence: |
|---|---|
| BE | BE |

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country.)*

LUYTEN, Frank
Baron d'Huartlaan 193
B-1950 Kraainem
Belgium

| State *(that is, country)* of nationality: | State *(that is, country)* of residence: |
|---|---|
| BE | BE |

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country.)*

DE BARI, Cosimo
18 Ethelbert Close
Bromley, Kent BR1 1JB
United Kingdom

| State *(that is, country)* of nationality: | State *(that is, country)* of residence: |
|---|---|
| IT | GB |

[X] Further applicants are indicated on a continuation sheet.



*See Notes to the demand form*

International application No.
PCT/BE2004/000117

**Continuation of Box No. II APPLICANT(S)**

*If none of the following sub-boxes is used, this sheet should not be included in the demand.*

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country.)*

DELL'ACCIO, Francesco
150 Farnaby Road
Bromley, Kent BR2 0BB
United Kingdom

| State *(that is, country)* of nationality: | State *(that is, country)* of residence: |
|---|---|
| IT | GB |

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country.)*

| State *(that is, country)* of nationality: | State *(that is, country)* of residence: |
|---|---|
| | |

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country.)*

| State *(that is, country)* of nationality: | State *(that is, country)* of residence: |
|---|---|
| | |

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country.)*

| State *(that is, country)* of nationality: | State *(that is, country)* of residence: |
|---|---|
| | |

☐ Further applicants are indicated on another continuation sheet.

International application No.
PCT/BE2004/000117

**Box No. III AGENT OR COMMON REPRESENTATIVE; OR ADDRESS FOR CORRESPONDENCE**

The following person is [X] agent [ ] common representative

and [X] has been appointed earlier and represents the applicant(s) also for international preliminary examination.

[ ] is hereby appointed and any earlier appointment of (an) agent(s)/common representative is hereby revoked.

[ ] is hereby appointed, specifically for the procedure before the International Preliminary Examining Authority, in addition to the agent(s)/common representative appointed earlier.

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country.)*

BIRD, William E.
Bird Goën & Co
Klein Dalenstraat 42A
B-3020 Winksele
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+32 16 48 05 62

Facsimile No.
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Teleprinter No.

Agent's registration No. with the Office

[ ] **Address for correspondence:** Mark this check-box where no agent or common representative is/has been appointed and the space above is used instead to indicate a special address to which correspondence should be sent.

**Box No. IV BASIS FOR INTERNATIONAL PRELIMINARY EXAMINATION**

**Statement concerning amendments:***

1. The applicant wishes the international preliminary examination **to start on the basis of:**

[X] the international application as originally filed

the description [X] as originally filed
[ ] as amended under Article 34

the claims [ ] as originally filed
[ ] as amended under Article 19 (together with any accompanying statement)
[X] as amended under Article 34

the drawings [X] as originally filed
[ ] as amended under Article 34

2. [ ] The applicant wishes any amendment to the claims under Article 19 to be considered as reversed.

3. [ ] The applicant wishes the start of the international preliminary examination **to be postponed** until the expiration of the applicable time limit under Rule 69.1(d).

4. [ ] The applicant expressly wishes the international preliminary examination **to start earlier** than at the expiration of the applicable time limit under Rule *54bis*.1(a).

* Where no check-box is marked, international preliminary examination will start on the basis of the international application as originally filed or, where a copy of amendments to the claims under Article 19 and/or amendments of the international application under Article 34 are received by the International Preliminary Examining Authority before it has begun to draw up a written opinion or the international preliminary examination report, as so amended.

**Language for the purposes of international preliminary examination:** ENGLISH

[X] which is the language in which the international application was filed.
[ ] which is the language of a translation furnished for the purposes of international search.
[ ] which is the language of publication of the international application.
[ ] which is the language of the translation (to be) furnished for the purposes of international preliminary examination.

**Box No. V ELECTION OF STATES**

The filing of this demand constitutes the election of all Contracting States which are designated and are bound by Chapter II of the PCT.

International application No.
PCT/BE2004/000117

**Box No. VI CHECK LIST**

The demand is accompanied by the following elements, in the language referred to in Box No. IV, for the purposes of international preliminary examination:

| | | | For International Preliminary Examining Authority use only: received | not received |
|---|---|---|---|---|
| 1. translation of international application | : | sheets | ☐ | ☐ |
| 2. amendments under Article 34 | : | 3 sheets | ☐ | ☐ |
| 3. copy (or, where required, translation) of amendments under Article 19 | : | sheets | ☐ | ☐ |
| 4. copy (or, where required, translation) of statement under Article 19 | : | sheets | ☐ | ☐ |
| 5. letter | : | 2 sheets | ☐ | ☐ |
| 6. other *(specify)* | : | sheets | ☐ | ☐ |

The demand is also accompanied by the item(s) marked below:

1. ☒ fee calculation sheet
2. ☐ original separate power of attorney
3. ☐ original general power of attorney
4. ☐ copy of general power of attorney; reference number, if any:
5. ☐ statement explaining lack of signature
6. ☐ sequence listing in computer readable form
7. ☐ tables in computer readable form related to a sequence listing
8. ☐ other *(specify)*:

**Box No. VII SIGNATURE OF APPLICANT, AGENT OR COMMON REPRESENTATIVE**

*Next to each signature, indicate the name of the person signing and the capacity in which the person signs (if such capacity is not obvious from reading the demand).*

William E. Bird

William E. Bird

**For International Preliminary Examining Authority use only**

1. Date of actual receipt of DEMAND:

2. Adjusted date of receipt of demand due to CORRECTIONS under Rule 60.1(b):

3. ☐ The date of receipt of the demand is AFTER the expiration of 19 months from the priority date and item 4 or 5, below, does not apply.
   ☐ The applicant has been informed accordingly.
4. ☐ The date of receipt of the demand is WITHIN the time limit of 19 months from the priority date as extended by virtue of Rule 80.5.
5. ☐ Although the date of receipt of the demand is after the expiration of 19 months from the priority date, the delay in arrival is EXCUSED pursuant to Rule 82.
6. ☐ The date of receipt of the demand is AFTER the expiration of the time limit under Rule 54*bis*.1(a) and item 7 or 8, below, does not apply.
7. ☐ The date of receipt of the demand is WITHIN the time limit under Rule 54*bis*.1(a) as extended by virtue of Rule 80.5.
8. ☐ Although the date of receipt of the demand is after the expiration of the time limit under Rule 54*bis*.1(a), the delay in arrival is EXCUSED pursuant to Rule 82.

**For International Bureau use only**

Demand received from IPEA on:



*See Notes to the demand form*

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**CHAPTER II**

# PCT

## FEE CALCULATION SHEET

**Annex to the Demand**

| | |
|---|---|
| International application No. | PCT/BE2004/000117 |
| Applicant's or agent's file reference | T2952-PCT |

For International Preliminary Examining Authority use only

Date stamp of the IPEA

Applicant

Tigenix N.V. et al.

### CALCULATION OF PRESCRIBED FEES

| | | |
|---|---|---|
| 1. Preliminary examination fee ..... | EUR 1.530,- P | |
| 2. Handling fee *(Applicants from certain States are entitled to a reduction of 75% of the handling fee. Where the applicant is (or all applicants are) so entitled, the amount to be entered at H is 25% of the handling fee.)* ..... | EUR 129,- H | |
| 3. Total of prescribed fees Add the amounts entered at P and H and enter total in the TOTAL box ..... | EUR 1.659,- TOTAL | |

### MODE OF PAYMENT

- [X] authorization to charge deposit account with the IPEA (see below)
- [ ] cheque
- [ ] postal money order
- [ ] bank draft
- [ ] cash
- [ ] revenue stamps
- [ ] coupons
- [ ] other *(specify)*:

### AUTHORIZATION TO CHARGE (OR CREDIT) DEPOSIT ACCOUNT

*(This mode of payment may not be available at all IPEAs)*

IPEA/ EP

- [X] Authorization to charge the total fees indicated above.
- [X] *(This check-box may be marked only if the conditions for deposit accounts of the IPEA so permit)* Authorization to charge any deficiency or credit any overpayment in the total fees indicated above.

Deposit Account No.: 28020053

Date: 1 June 2005

Name: William E. Bird

Signature: William E. Bird



*See Notes to the fee calculation sheet*

Amended claims for PCT/BE2004/000117 (version showing amendments)

1. Use of CXCL6 for the preparation of a medicament for the promotion of cartilage and/or bone formation in vivo.

2. The use according to claim 1, in the prevention or treatment of a cartilage or osteochondral defect.

3. The use according to claim 1 or 2, wherein the source of CXCL6 is a population of CXCL6 expressing cells.

4. The use according to any of claims 1 to 3, wherein the said CXCL6 is recombinant or synthetic.

5. The use according to any of claims 1 to 4, wherein said CXCL6 is administered through gene therapy.

6. The use according to any of claims 1 to 5, wherein said CXCL6 is administered to the osteochondral defect in a gradient.

7. The use according to any one of claims 1 to 6, wherein said medicament further comprises chondrogenic cells or precursor cells thereof.

8. The use according to claim 7, wherein said precursor cells are isolated from synovial membrane.

9. Use of CXCL6-expressing cells for the preparation of a medicament for the promotion of formation of cartilage or bone in vivo wherein said cells comprise a foreign DNA encoding said CXCL6, under control of a promoter.

10. The use according to claim 9, for the prevention or treatment of a cartilage or osteochondral defect.

11. The use according to claim 9 or 10, wherein said CXCL6-expressing cells are chondrogenic cells.

12. The use according to claim 11, wherein said chondrogenic cells are isolated from connective tissue.

~~13. The use according to claim 11 or 12, wherein said chondrogenic cells comprise a foreign DNA encoding said CXCL6, under control of a promoter.~~

~~14.~~13. The use according to any one of claims ~~10~~9 to ~~13~~12, wherein said CXCL6- expressing cells are embedded in a matrix.

~~15. A composition for use as a medicament comprising a cell population or cells expressing CXCL6.~~

~~16. The composition according to claim 15, wherein said CXCL6 expressing cells or cell population are chondrogenic.~~

~~17. A composition according to claim 15 or 16, wherein said cells or cell population are embedded in a suitable pharmaceutical carrier.~~

~~18.~~ 14. Use according to any one of claims 1 to ~~14~~13, wherein the cartilage defect is a joint surface defect not related to inflammation.

~~19.~~15. Use according to claim ~~18~~14, wherein said joint surface defect occurs in the context of osteoarthritis.

~~20. Use of a compound inducing the expression of CXCL6 for the preparation of a medicament for the promotion of cartilage or bone formation in vivo.~~

~~21.The use according to claim 20, for the treatment or prevention of cartilage or osteochondral defects.~~

~~22.The use according to claim 20 or 21, wherein said compound induces expression of CXCL6 in chondrogenic cells.~~

~~23.~~16. The use of expressed CXCL6 as a marker for chondrocyte phenotypic stability.

~~24~~17. The use of CXCL6 for the promotion of cartilage and/or bone formation in vitro.

~~25.A method of modulating the differentiation of a progenitor cell population into a cartilage-producing cell population, said method comprising administering to said progenitor cell population a ligand or inhibitor of the CXCR1 or CXCR2 receptor.~~

~~26.The method according to claim 25, which comprises inhibiting said differentiation of a progenitor cell population into a cartilage-producing cell population using an inhibitor of CXCR1 or CXCR2.~~

~~27.~~18. A method of inducing or restoring chondrocyte phenotypic stability in a progenitor cell population, said method comprising the step of administering CXCL6 to said progenitor cell population.

~~28.~~19. A method of inducing or restoring differentiation of a precursor cell population into__--chondrocytes, said method comprising the step of administering CXCL6 to said precursor cell population.

~~29.~~20. A method for the ~~detection of a~~preparation of a pharmaceutical comprising a compound or mixture of compounds for the promotion of

cartilage and bone promotion in vivo, said compound or mixture of compounds modulating CXCL6 signalling, and said method comprising the steps of:

- contacting a cell population with a candidate compound or mixture of compounds, and
- determining ~~a modified~~an increased expression level of CXCL6.
- Identifying said compound or mixture of compounds as a compound capable of promoting cartilage and bone formation in vivo
- Preparing a pharmaceutical composition comprising said compound or mixture of compounds

~~30.~~21. The method according to claim ~~29~~20 wherein the cell population is ~~being~~ selected from the group consisting of chondrocytes, chondrocytes precursors and chondrocyte progenitors.

~~31.~~22. The method according to claim ~~30~~21 further comprising the step of determining one or more morphological or molecular parameters of said chondrocyte, chondrocyte precursor or chondrocyte progenitor cell population.

23. A method for producing a medicament for the promotion of formation of cartilage or bone in vivo, which method comprises
- obtaining cells from a small cartilage biopsy
- selecting cells therefrom based on CXCL6 expression

Amended claims for PCT/BE2004/000117 (clean copy)

1. Use of CXCL6 for the preparation of a medicament for the promotion of cartilage and/or bone formation in vivo.

2. The use according to claim 1, in the prevention or treatment of a cartilage or osteochondral defect.

3. The use according to claim 1 or 2, wherein the source of CXCL6 is a population of CXCL6 expressing cells.

4. The use according to any of claims 1 to 3, wherein the said CXCL6 is recombinant or synthetic.

5. The use according to any of claims 1 to 4, wherein said CXCL6 is administered through gene therapy.

6. The use according to any of claims 1 to 5, wherein said CXCL6 is administered to the osteochondral defect in a gradient.

7. The use according to any one of claims 1 to 6, wherein said medicament further comprises chondrogenic cells or precursor cells thereof.

8. The use according to claim 7, wherein said precursor cells are isolated from synovial membrane.

9. Use of CXCL6-expressing cells for the preparation of a medicament for the promotion of formation of cartilage or bone in vivo wherein said cells comprise a foreign DNA encoding said CXCL6, under control of a promoter.

10. The use according to claim 9, for the prevention or treatment of a cartilage or osteochondral defect.

11. The use according to claim 9 or 10, wherein said CXCL6-expressing cells are chondrogenic cells.

12. The use according to claim 11, wherein said chondrogenic cells are isolated from connective tissue.

13. The use according to any one of claims 9 to 12, wherein said CXCL6-expressing cells are embedded in a matrix.

14. Use according to any one of claims 1 to 13, wherein the cartilage defect is a joint surface defect not related to inflammation.

15. Use according to claim 14, wherein said joint surface defect occurs in the context of osteoarthritis.

16. The use of expressed CXCL6 as a marker for chondrocyte phenotypic stability.

17. The use of CXCL6 for the promotion of cartilage and/or bone formation in vitro.

18. A method of inducing or restoring chondrocyte phenotypic stability in a progenitor cell population, said method comprising the step of administering CXCL6 to said progenitor cell population.

19. A method of inducing or restoring differentiation of a precursor cell population into chondrocytes, said method comprising the step of administering CXCL6 to said precursor cell population.

20. A method for the preparation of a pharmaceutical comprising a compound or mixture of compounds for the promotion of cartilage and bone promotion

in vivo, said compound or mixture of compounds modulating CXCL6 signalling, and said method comprising the steps of:

- contacting a cell population with a candidate compound or mixture of compounds, and
- determining an increased expression level of CXCL6.
- Identifying said compound or mixture of compounds as a compound capable of promoting cartilage and bone formation in vivo
- Preparing a pharmaceutical composition comprising said compound or mixture of compounds

21. The method according to claim 20 wherein the cell population is selected from the group consisting of chondrocytes, chondrocyte precursors and chondrocyte progenitors.

22. The method according to claim 21 further comprising the step of determining one or more morphological or molecular parameters of said chondrocyte, chondrocyte precursor or chondrocyte progenitor cell population.

23. A method for producing a medicament for the promotion of formation of cartilage or bone in vivo, which method comprises

- obtaining cells from a small cartilage biopsy
- selecting cells therefrom based on CXCL6 expression